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P-glycoprotein inhibitors stimulate renal phosphate reabsorption in rats

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Background. Dipyridamole (Dip) was previously shown to increase renal phosphate (Pi) reabsorption in humans. However, the mechanism(s) underlying this renal tubular effect is not fully elucidated. It is known that Dip inhibits the activity of the P-glycoprotein (Pgp) multidrug resistance protein 1 (MDR1) expressed on the apical membrane of renal proximal tubular cells where the Na-Pi cotransporter (NPT2) is also expressed. We hypothesized that Dip could increase renal Pi reabsorption by inhibiting Pgp activity.

Methods. To test this hypothesis, the effects of Dip, verapamil (Ver), and cyclosporine A (CsA), three unrelated Pgp inhibitors, were studied on the renal Pi reabsorption in rats.

Results. All three drugs decreased the fractional excretion of Pi (FE_{Pi}) in a dose-dependent manner within one hour after beginning the drug infusion, without altering the glomerular filtration rate or serum parathyroid hormone concentration. Sodium-dependent Pi uptake but not Na-glucose transport was increased in brush-border membrane vesicles (BBMVs) when comparing treated with untreated rats. Western blot analysis showed that NPT2 protein was increased in BBMVs from treated rats. Dip and Ver had no effect when applied directly to BBMVs prepared from untreated rats. Pretreatment of rats with colchicine prevented the effects of Dip on the FE_{Pi} and NPT2 expression in brush-border membranes.

Conclusions. Our results suggest that inhibition of Pgp in the proximal tubule increases Pi uptake and NPT2 translocation to the apical membrane.

Phosphate (Pi), one of the most abundant anions in humans, plays a central role in cellular metabolism and bone mineralization. Pi homeostasis is controlled by the kidney, which adapts urinary Pi excretion to Pi uptake in order to keep serum Pi in a normal range [1, 2]. Pi is mainly reabsorbed in the proximal tubule through the type 2 sodium-phosphate cotransporter (NPT2). This transporter is exclusively detected in the brush border of proximal tubular cells [3] and transports approximately 80% of the Pi reabsorbed in the kidney [4]. In vivo, hormonal modulation of renal Pi reabsorption [for example, by parathyroid hormone (PTH) or dopamine] has been ascribed to changes in activity and/or expression of NPT2. In pathology, NPT2 expression is decreased in mice and probably in humans, with X-linked hypophosphatemia due to mutations in the *PHEX* gene [5, 6].

An impairment of renal reabsorption of Pi leads to hypophosphatemia with high urinary Pi excretion that may induce bone demineralization, hypervitaminosis D, hypercalciuria, and urolithiasis [4, 7–11]. We have recently shown that dipyridamole (Dip) increases renal Pi reabsorption and serum phosphorus in patients with hypophosphatemia resulting from idiopathic renal Pi leak [12]. The mechanism whereby Dip stimulates renal Pi reabsorption in vivo is not fully understood. This drug has multiple pharmacological properties and cellular targets. Particularly, Dip inhibits cellular adenosine uptake [13, 14] and P-glycoprotein (Pgp) activity [15]. The Pgps are encoded by the multidrug resistance genes. One of these proteins, multidrug resistance protein 1 (MDR1) Pgp, is expressed in the apical membrane of renal proximal tubular cells [16]. It is a 170 kD protein belonging to the ATP binding cassette (ABC) superfamily, which can extrude a wide variety of hydrophobic amphipathic compounds from proximal cells to urine [17, 18]. The members of the ABC family are thought to be regulatory agents. Hence, MDR1 Pgp has been shown to activate a chloride channel [19]. Since on the one hand Na-Pi cotransporters and MDR1 Pgp are both expressed in the brush border membrane of proximal tubular cells and on the other hand ABC family members may be regulatory factors, we hypothesized that Dip-induced stimulation of renal Pi reabsorption may result from the inhibition of MDR1 Pgp. Since MDR1 Pgp activity cannot be mea-

Key words: transport, phosphorus, sodium-Pi cotransport, cellular metabolism, bone mineralization, type 2 Na-Pi cotransporter, hypophosphatemia.

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sured in proximal tubule in vivo, the stimulation of Pi reabsorption and inhibition of MDR Pgp activity induced by Dip cannot be directly correlated. To circumvent this problem, we compared the effects of Dip and two other inhibitors of MDR1 Pgp activity [verapamil (Ver), cyclosporine A (CsA)] on renal Pi reabsorption in rats. We measured urinary Pi excretion in vivo and NPT2 expression in brush-border membranes from proximal tubules. This study shows that all three Pgp inhibitors increased renal Pi reabsorption through increased expression of NPT2 in brush-border membranes, suggesting that Pgp modulates renal Pi transport.

METHODS

Experimental animals, clearance studies

Experiments were performed with four- to six-week-old male Sprague-Dawley rats. The animals were fed a 0.6% Pi diet. Tritiated inulin clearance studies were carried out on anesthetized animals as previously described [20]. Inulin clearance measurements were made twice during periods without drugs (basal periods) and after drug infusion (experimental periods). Dip was from Boehringer Ingelheim France (Paris, France). Ver was from Laboratoire Knoll (Levallois Perret, France), and CsA was from Sigma Chemical Co. (St. Louis, MO, USA). All drugs were diluted in isotonic saline and, after the control periods, infused throughout the duration of experiments. Dip was infused at a maximal rate of 25 μg/min/100 g body weight, Ver at 7.5 μg/min/100 g body weight, and CsA at 50 μg/min/100 g body weight. In a set of experiments, rats were treated with colchicine (100 μg/100 g body weight), dissolved in isotonic saline, and administered via intraperitoneal injection three hours before Dip infusion. This dose of colchicine has been shown to disrupt the microtubule network [21]. Serum and urinary phosphorus concentrations were determined using a standard method (Phosphore UV; Merck Clevenot, Cheneviere les Louvres, France). Serum PTH concentration determination was made with an immunoradiometric kit assay (Nichols Institute Diagnostics, Immutopics Inc., San Clemente, CA, USA).

Preparation of brush-border membrane vesicles

At the end of the experiments, blood was drawn from the aorta, and the kidneys were rapidly removed. Thin slices of cortex were cut at 4°C and homogenized with a Polytron in a buffer consisting of (mmol/L): 300 DL-mannitol, 5 egtazic acid (EGTA), 0.5 phenylmethylsulfonyl fluoride, and 16 HEPES (pH 7.5 adjusted with Tris). Brush-border membrane vesicles (BBMVs) were obtained from this homogenate by Mg^{2+} precipitation as described by Levi, Jameson, and Van der Meer [22]. The final pellet was resuspended in a buffer consisting of 300 mmol/L mannitol and 16 mmol/L HEPES-Tris, pH 7.5.

Na-dependent transport measurements

The uptakes of Pi and α-methyl glucopyranoside were performed on freshly isolated BBMVs. Uptakes were performed in a buffered solution containing (mmol/L) 150 NaCl, 16 HEPES-Tris (pH 7.5). Na-free solutions were made iso-osmotic by replacing NaCl with mannitol 300 mmol/L. The BBMVs were incubated for five seconds in the presence of 0.1 mmol/L KH_2PO_4 mixed with [^{32}P] orthophosphate (0.5 μCi/mL) or of 1 mmol/L α-methyl glucopyranoside mixed with [^{14}C] α-methyl glucopyranoside (1 μCi/mL). The uptake was stopped by the addition of 1 mL of ice-cold solution consisting of (mmol/L) 300 mannitol, 10 HEPES-Tris (pH 7.5), 2 $CaCl_2$, and 10 Na arsenate. The BBMV suspensions were then applied to 0.45 μm size pore filters under vacuum conditions. Filters were washed twice with 3 mL of ice-cold stop solution and processed for liquid scintillation counting. Na-dependent uptakes were calculated by subtracting uptake values measured in Na-free solutions from those obtained in the presence of Na. Protein concentrations were measured by the method of Bradford [23]. Uptake values were corrected for protein content.

Western blot

Aliquots of BBMV were denaturated for five minutes at 95°C in 2% sodium dodecyl sulfate (SDS), 10% glycerol, 0.5 mmol/L ethylenediaminetetraacetic acid (EDTA), and 95 mmol/L Tris-HCl, pH 6.8. Ten micrograms of BBMV proteins were loaded per lane and separated on 10% polyacrylamide gel according to the method of Laemmli [24] and electrotransferred on nitrocellulose membranes. After blockage with 5% fat-free milk powder and 1% Triton X-100 in Tris-buffered saline (20 mmol/L, pH 7.3), the membranes were incubated with the primary antibody against NPT2 [24] or β-actin (Sigma Immunochemical Co.) at a final dilution of 1:5000 and 1:10,000, respectively. The primary antibody binding was visualized by chemiluminescence using the ECL Western blotting detection system (Amersham, les Ulis, France). Quantification was made using the NIH imager version 1.6 software (Bethesda, MD, USA).

Statistical analysis

Fractional excretion of Pi (FE_{Pi}) values were compared using an analysis of variance (ANOVA) for repeated measures followed by a PLSD Fisher's test. Other comparisons were made using a two-way ANOVA and a PLSD Fisher's test when allowed by *F* values. Results are presented as mean ± SE.

RESULTS

Effects of Pgp inhibitors on glomerular filtration rate

Because renal Pi excretion can be affected by variations of glomerular filtration rate (GFR), we first determined

Table 1. Effect of dipyridamole (Dip), verapamil (Ver) or cyclosporine A (CsA) on glomerular filtration rate (GFR)

	GFR		*P* values
	Basal periods	Experimental periods	experimental vs. basal
Control	1.40 ± 0.04	1.45 ± 0.06	NS
Dip 18 μg/min/100 g	1.47 ± 0.02	1.43 ± 0.05	NS
Dip 25 μg/min/100 g	1.36 ± 0.05	1.39 ± 0.09	NS
Ver 5 μg/min/100 g	1.48 ± 0.04	1.31 ± 0.08	NS
Ver 7.5 μg/min/100 g	1.33 ± 0.05	1.26 ± 0.09	NS
CsA 30 μg/min/100 g	1.46 ± 0.07	1.6 ± 0.02	NS
CsA 50 μg/min/100 g	1.52 ± 0.16	1.40 ± 0.15	NS

GFR was measured before (basal) and after (experimental) infusion of different doses of drugs or vehicle alone (control). GFR is expressed as mL/min. The drugs did not modify GFR. NS is not significant.

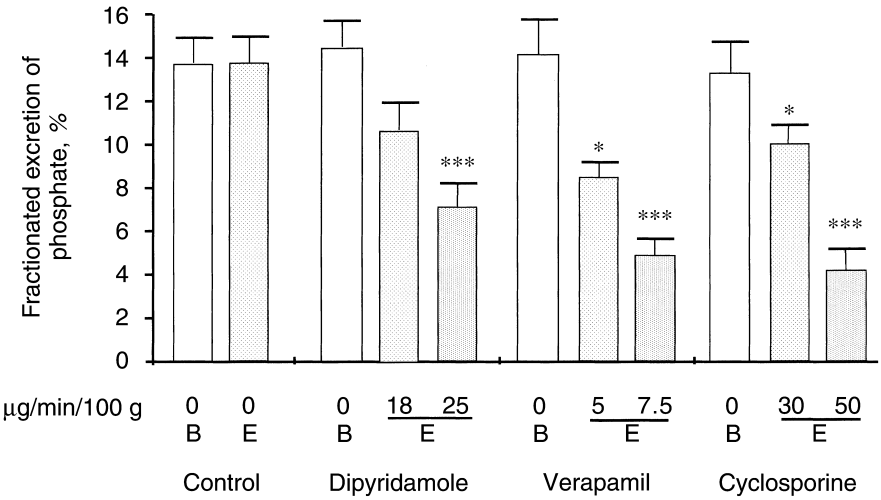

Fig. 1. Effects of dipyridamole, verapamil, and cyclosporine A on fractional excretion of phosphate in rats. Rats were infused with vehicle alone (control), dipyridamole (18 or 25 μg/min/100 g), verapamil (5 or 7.5 μg/100 g/min), or cyclosporine A (30 or 50 μg/min/100 g), as described in the **Methods** section. Fractionated excretion of phosphate was measured before (basal: B) and after (experimental: E) infusion of drugs. Dipyridamole, verapamil, and cyclosporine significantly decreased fractionated excretion of phosphate. There was no difference between drugs. $*P < 0.05$; $***P < 0.001$ compared with basal values ($N = 10$).

the maximal doses of Dip, Ver, and CsA that could be infused to rats without altering GFR. GFR was assessed during basal periods and after infusion of the drugs or the vehicle alone (control). GFR remained unchanged for infusion rates up to 25 μg/min/100 g body weight, 7.5 μg/min/100 g body weight, and 50 μg/min/100 g body weight for Dip, Ver, and CsA, respectively (Table 1). These infusion rates (Dip, 25 μg/min/100 g body weight; Ver, 7.5 μg/min/100 g body weight; CsA, 50 μg/min/100 g body weight) were used for all subsequent experiments except for the study of the dose effect of the drug on FE_{Pi} (discussed later in this article).

Effects of Pgp inhibitors on fractional excretion of Pi

First, the effects of Dip, Ver, and CsA infusion on the FE_{Pi} were studied. FE_{Pi} was measured before (basal) and after (experimental) infusion of the drug or the vehicle. Figure 1 shows that basal values of FE_{Pi} were similar in the four groups of rats. However, after the drug treatment, FE_{Pi} significantly decreased in all groups except in controls (Fig. 1). The effect of Dip, Ver, and CsA on FE_{Pi} was dose dependent, as shown in Figure 1. At the maximal infusion rates used, the decrease of FE_{Pi} was not significantly different between the drugs and ranged from 51 (25 μg/min/100 g body weight of Dip) to 66% (50 μg/min/100 g body weight of CsA). As shown in Figure 2, the decrease of FE_{Pi} induced by the three drugs occurred within one hour after beginning the drug infusion and was maximal after 90 minutes. The kinetic of inhibition was similar for the three drugs (Fig. 2). Serum phosphorus was slightly but not significantly increased by drug infusion (Table 2).

Lack of modification of serum PTH concentrations

In vivo, a major determinant of FE_{Pi} is the serum PTH level. We measured serum PTH values after infusion of Dip, Ver, or vehicle alone (control). Drug infusion did not modify serum PTH concentrations when compared with control (serum PTH in controls, 23.7 ± 1.5; Dip, 24.3 ± 3.3; and Ver, 24.0 ± 4.2 pg/mL; mean ± SEM, $N = 3$).

Effect of Pgp inhibitors on phosphate and glucose uptake

The first and limiting step of Pi reabsorption in the proximal tubule is the transport through apical membranes where Na-Pi cotransporters are expressed. To eval-

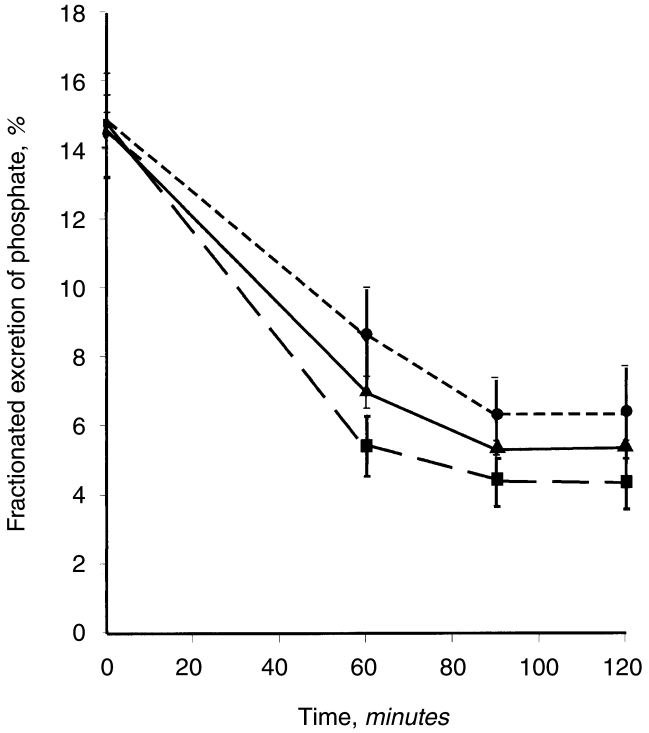

Fig. 2. Time effects of P-glycoprotein (PgP) inhibitors on fractionated excretion of phosphate. Time 0 indicates the beginning of drug infusion. Drugs were infused over one hour. At the end of infusion, FE_{Pi} had significantly decreased for the three drugs, and the curves reached a plateau within 90 minutes, which was maintained for up to 120 minutes ($N = 10$). Symbols and infusion rates of drugs are: (●) dipyridamole 25 μg/min/100 g; (■) verapamil 7.5 μg/min/100 g; (▲) cyclosporine 50 μg/min/100 g.

uate whether the increase of Pi reabsorption was due to a primary modification of the apical transport, Pi uptake was measured in BBMVs prepared from rats treated either by Dip, Ver, or vehicle alone. Sodium-dependent Pi uptake was significantly higher (40% increase) in BBMVs from rats treated by Pgp inhibitors as compared with controls, while sodium-independent Pi uptake was not significantly altered by drug treatment (Table 3). We also measured BBMV Na-dependent α-methylglucopyranoside uptake. The results showed that neither the Dip nor Ver treatment modified glucose uptake (Table 3), indicating that the drugs specifically stimulated Pi transport.

To determine whether the effect of Pgp inhibitors on Pi uptake resulted from a direct interaction between these drugs and Na-Pi cotransporters, Dip, or Ver were added in vitro to BBMV obtained from untreated rats (Fig. 3). Under these conditions, Na-dependent Pi uptake was not modified, suggesting that the drugs could not alter the activity of the Pi transporters directly.

Effect of Pgp inhibitors on NPT2 abundance and distribution

Approximately 80% of Pi is reabsorbed in the proximal tubule through the NPT2. To determine whether the increase in Na-dependent uptake demonstrated in BBMV prepared from Pgp inhibitor-treated rat kidneys was associated with an increase in NPT2 expression, Western blotting was used to compare the NPT2 protein abundance in BBMVs from control rats and rats treated by Dip, Ver, or CsA. The drug infusion led to a 50% increase of NPT2 protein in BBMV with no difference between drugs (Fig. 4); however, NPT2 protein abundance in cortical homogenate was not modified by Dip treatment (Fig. 5).

The effects of these drugs on NPT2 protein expression were compared to those observed after an acute suppression of PTH secretion by parathyroidectomy. Under these conditions, urinary Pi excretion became undetectable within 30 minutes. Two hours after parathyroidectomy, the kidneys were removed, and BBMVs were prepared to measure NPT2 expression by Western blotting. In parathyroidectomized rats, NPT2 expression was increased by 84% by comparison with sham-operated rats (data not shown).

Table 2. Effect of drug infusion on serum phosphorus concentrations

	Serum phosphorus *mmol/L*	
	Basal	Experimental
Control	2.46 ± 0.09	2.41 ± 0.08
Dipyridamole *25 μg/min/100 g*	2.51 ± 0.1	2.69 ± 0.08
Verapamil *7.5 μg/min/100 g*	2.49 ± 0.11	2.66 ± 0.12

Serum phosphorus was determined before and two hours after beginning the drug infusion. The differences did not reach significance (ANOVA for repeated measures). The results are expressed as the mean ± SE, $N = 7$.

Consequences of colchicine treatment on Dip effects

To determine whether the increase in NPT2 expression evidenced in Pgp inhibitor-treated rats was dependent on the integrity of the microtubule network, the influence of colchicine on the antiphosphaturic effect of Dip was evaluated at doses known to disrupt the microtubule network in the kidney [21]. Colchicine pretreatment of the rats prevented the decrease of FE_{Pi} induced by Dip (Fig. 6). In addition, NPT2 protein expression in BBM from Dip + colchicine-treated rat was lower than that measured in rat treated with Dip alone (Fig. 7).

DISCUSSION

This work shows that three dissimilar and well-known inhibitors of Pgp activity—Dip, Ver, and CsA—decreased renal FE_{Pi} and increased Na-dependent Pi transport in BBMV and NPT2 expression in the apical membrane of proximal tubular cells.

Parathyroid hormone is known to inhibit Pi reabsorption by the renal proximal tubule. However, PTH did not mediate any drug effect on Pi reabsorption, since the serum PTH concentration was not modified follow-

Table 3. Phosphate (Pi) and glucose uptake in brush-border membrane vesicles (BBMV) from rats treated by dipyridamole (25 μg/min/100 g), verapamil (7.5 μg/min/100 g) or saline solution (control)

	Na-dependent Pi uptake *pmol Pi/5 s/mg protein*	Na-independent Pi uptake *pmol Pi/5 s/mg protein*	Na-dependent glucose uptake *nmol glucose/5 s/mg protein*
Control	407.5 ± 9.7	115.8 ± 17.1	433.6 ± 66
Dipyridamole	563.8 ± 29.7[a]	125.8 ± 11.8	436.1 ± 58.1
Verapamil	560.0 ± 12.1[a]	126.0 ± 6.8	435.7 ± 109.7

$N = 4$. Pgp inhibitors increased Na-dependent Pi uptake but did not modify Na-dependent glucose transport. Na-independent Pi uptake was not modified by the dipyridamole or verapamil treatment.
[a] $P < 0.005$, ANOVA and PLSD Fisher's test

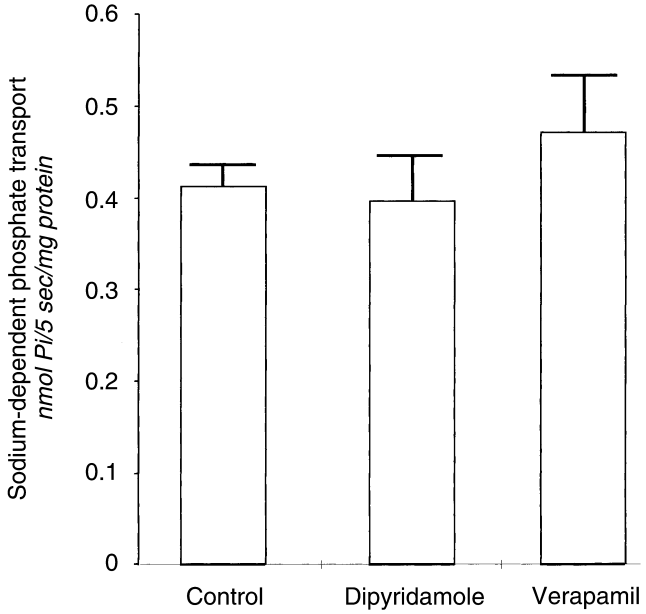

Fig. 3. Effect of drugs directly added to brush-border membrane vesicles (BBMV). BBMVs from untreated rats were incubated with vehicle alone (control), dipyridamole (80 μmol/L), or verapamil (0.1 μmol/L) for 30 minutes, and then Na-dependent Pi uptake was measured. Pi uptake was not modified by PgP inhibitors in this experimental condition ($N = 4$).

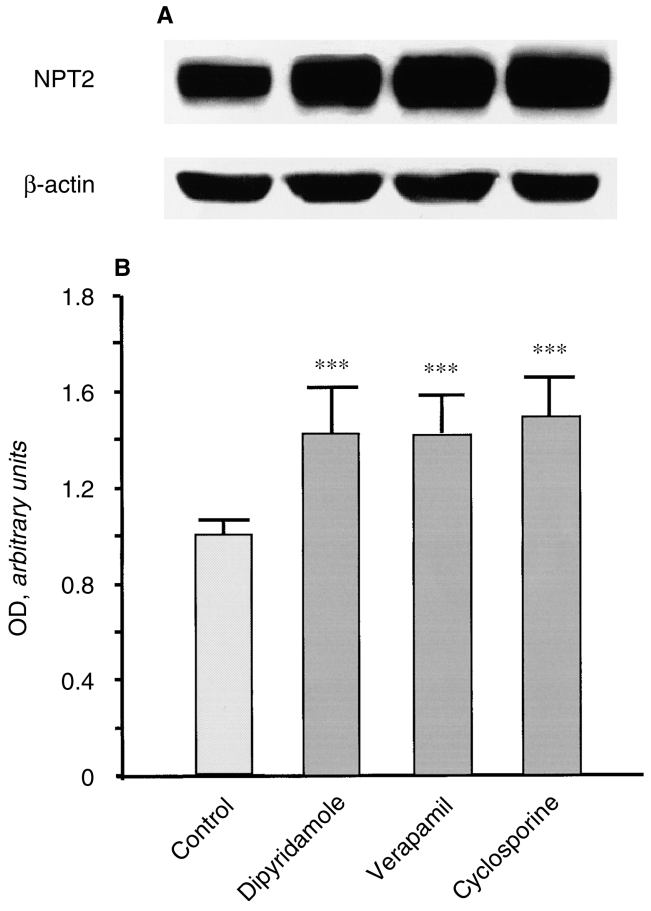

Fig. 4. Type 2 sodium-phosphate transporter (NPT2) protein expression in BBMV from rats treated by vehicle (control) or by dipyridamole (25 μg/min/100 g), verapamil (7.5 μg/min/100 g), or cyclosporine (50 μg/min/100 g). BBMVs were prepared as described in the **Methods** section. NPT2 was detected using a specific antibody. (*A*) Representative Western blot in BBMVs from different rats. (*B*) Results of the different experiments are expressed as arbitrary OD units, normalized for β actin density. NPT2 protein abundance was significantly higher in rats treated by Pgp inhibitors. ***$P < 0.001$ ($N = 4$).

ing drug infusion. These results are in agreement with our previous study in humans in which Dip increased renal Pi reabsorption and serum phosphorus in patients with hypophosphatemia and renal Pi leak with no change in serum PTH concentration [12]. Furthermore, that study demonstrated that Dip treatment did not modify urinary cAMP values [12], suggesting that the effect of Dip on FE_{Pi} was not due to a modification of cAMP synthesis by the proximal tubule.

The increase of Pi transport in vivo following drug infusion cannot be attributed to a decrease in intracellular Na concentration, which would stimulate the Na-dependent processes, because (*1*) it persisted in BBMV in which the gradient of Na is imposed and (*2*) these drugs did not affect Na-dependent glucose uptake.

Verapamil's effect on renal Pi reabsorption is similar to Dip. Ver is a calcium-channel blocker that differs from other calcium-channel blockers by its capacity to inhibit MDR1 Pgp activity [25]. Few studies describe the effect of calcium-channel blockers on renal Pi excretion. Lupinacci et al examined the effects of nitrendipine on FE_{Pi} in normal and hypertensive subjects and found that this drug, which does not inhibit Pgp activity, increased

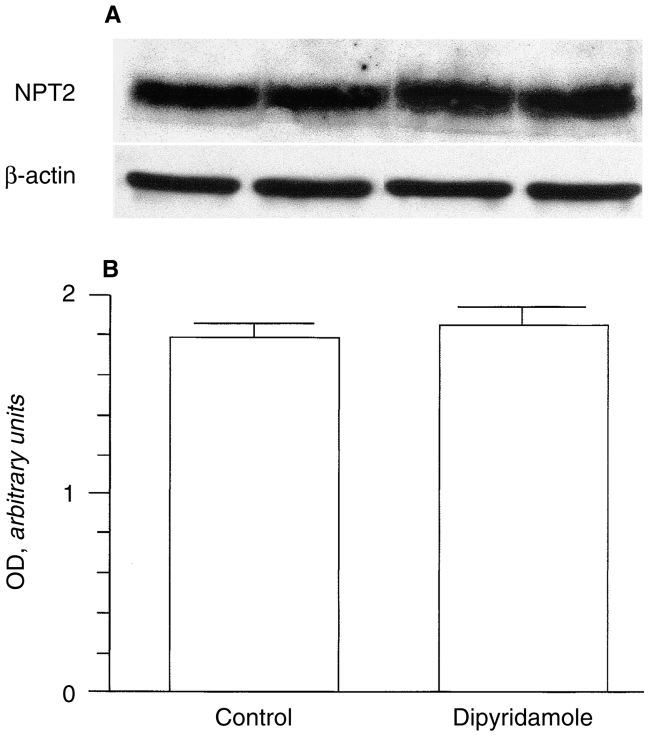

Fig. 5. NPT2 protein abundance in renal cortical homogenates from rats treated by vehicle (control) or dipyridamole (25 μg/min/100 g). (*A*) Representative Western blot in renal cortical homogenates from different rats. (*B*) Results of the different experiments ($N = 4$) are expressed as arbitrary OD units, normalized for β actin density. NPT2 protein expression was not significantly different in controls and dipyridamole-treated rats.

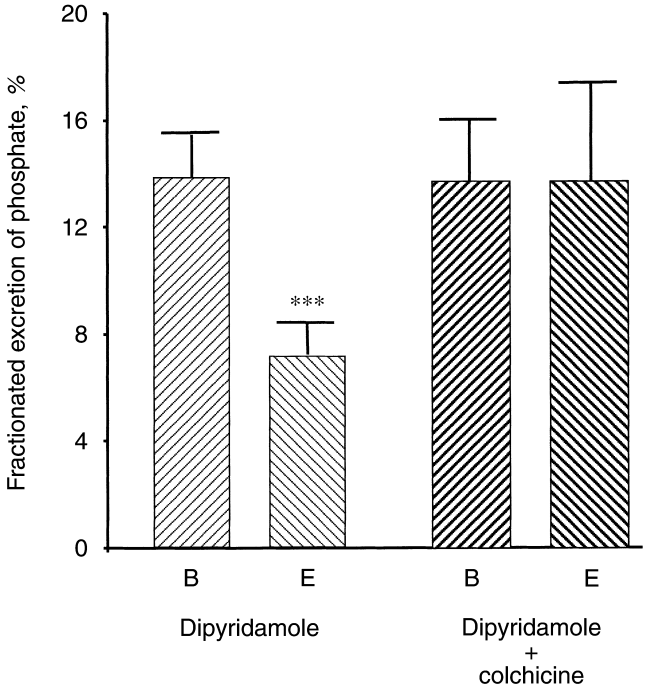

Fig. 6. Pretreatment of rats by colchicine prevents the decrease in FE_{Pi} following dipyridamole infusion (25 μg/min/100 g). Rats were treated by colchicine (0.1 mg/100 g body weight) intraperitoneally three hours before dipyridamole infusion. FE_{Pi} was compared before (B) and after dipyridamole infusion (E) in each rat. Dipyridamole infused alone decreased FE_{Pi}, and colchicine treatment abolished the effects of dipyridamole on FE_{Pi}.

FE_{Pi} [26]. These results suggest that the calcium-channel blocker properties cannot explain the Ver-induced decrease of FE_{Pi}. Two reports describe an effect of Ver on renal Pi reabsorption. In a clinical study, Sjöden et al found that Ver significantly increased serum phosphorus in patients with postsurgical hypoparathyroidism [27]. The authors did not measure the tubular transport maximum of Pi (TmPi), but this value can be estimated from the data given in the article. Using two methods, the nomogram of Walton and Bijvoet [28] and the equation defined by Kenny and Glen [29], the average value of TmPi was higher in patients treated with Ver.

In the opossum kidney epithelial cell line, Abraham, McAteer, and Kempson showed that in the presence of insulin, Ver increased Pi uptake [30].

Cyclosporine A is an immunosuppressive drug and a competitive inhibitor of MDR1 Pgp that reproduces the effect of Dip on FE_{Pi}. In the cytoplasm, CsA binds to cyclophilin. The CsA-cyclophilin complex binds to the β subunit of calcineurin, preventing calcineurin-phosphatase activity from being induced by an increase in cytosolic Ca [31, 32]. Calcineurin activity is present in the proximal tubule [31], and at high concentrations, CsA inhibits Na,K-ATPase activity in this tubular segment [31] probably by preventing Na,K-ATPase dephosphorylation. The expected consequence of a diminished Na,K-ATPase activity is the decrease of the Na-dependent uptakes. In the current study, renal Pi reabsorption was increased but the glucose uptake was not modified. These findings indicate that the effect of CsA cannot be attributed to an inhibition of Na,K-ATPase activity. Furthermore, an increase in Pgp expression [33] and a specific decrease in Pi uptake in renal BBMV [34] have been demonstrated following several days of CsA treatment in the rats. These results suggest that Pgp may modulate renal Pi uptake. In humans, Palestine, Austin, and Nussenblatt measured the renal Pi threshold (TmPi) in patients treated with CsA during 180 days for immune uveitis and found no significant change in TmPi [35]. However the authors did not study the short-term effects of CsA on TmPi, that is, before inducing Pgp expression. Thus, although each compound has specific properties in addition to its ability to inhibit PgP activity, the data from our present study and the literature all support the conclusion that the effects of the compounds on FE_{Pi} are associated with their capacity to inhibit PgP.

The Dip-, Ver-, and CsA-induced decrease in FE_{Pi} (Fig. 1) was associated with a specific increase in Na-dependent Pi uptake in BBMV (Table 3) and in NPT2

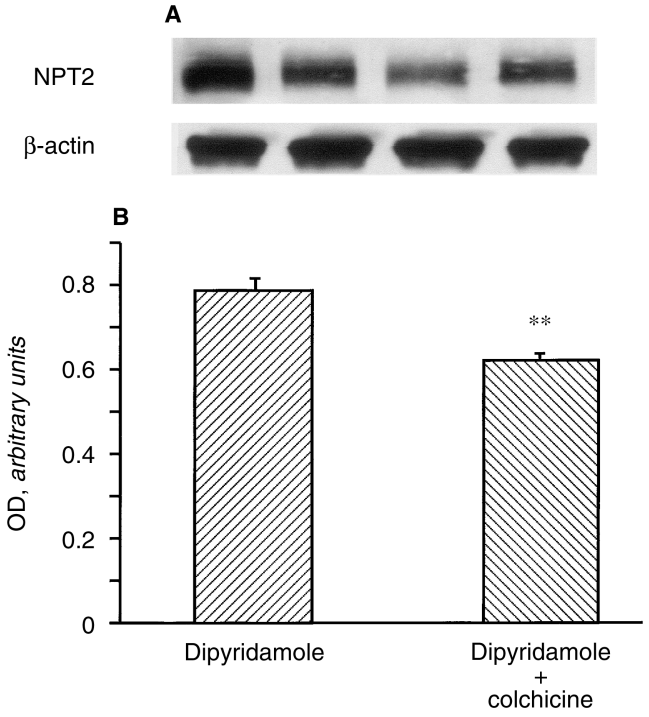

Fig. 7. Pretreatment of rats by colchicine prevents the increase of NPT2 expression in BBM following dipyridamole infusion (25 μg/min/100 g). Rats were treated by colchicine (0.1 mg/100 g body weight) intraperitoneally three hours before dipyridamole infusion. BBMs were prepared as described in the **Methods** section. NPT2 was detected using a specific antibody. (*A*) Representative Western blot in BBMV from different rats. (*B*) Results of the different experiments are expressed as arbitrary OD units, normalized for β actin density. NPT2 protein expression was significantly lower in BBM from rats treated by colchicine and dipyridamole compared with that in rats treated by dipyridamole alone (**$P = 0.007$, $N = 6$, mean ± SEM).

protein expression in BBM of proximal tubular cells (Fig. 4). Thus, the alteration of renal handling of Pi is related to a modification of the NPT2 protein content in brush border membrane. The drugs augment Pi transport in BBMV by approximately 40%, a finding that is consistent with the 50% increase of NPT2 expression and the 50% decrease of FE_{Pi}. Data obtained from knockout mice show that NPT2 reabsorbs more than 80% of the Pi filtrated by the glomeruli [4], indicating that the increase of NPT2 expression is likely to account for the decrease of FE_{Pi}.

The lack of effect of Pgp inhibitors added to the BBMV preparation suggests that Pgp does not interact directly with NPT2, but rather modulates a factor implicated in NPT2 expression. We cannot rule out, however, that the drugs must enter the proximal tubular cells to interact with NPT2.

P-glycoprotein inhibitors increased NPT2 protein expression in brush border membrane (Fig. 4) but not in cortical homogenate. The effect of Dip was prevented by colchicine treatment (Figs. 6 and 7), demonstrating that an intact microtubule network was mandatory for Dip to have an effect. These results suggest that Dip, and most likely Ver and CsA, decrease FE_{Pi} by stimulating NPT2 translocation in BBM.

We could not correlate the inhibition of Pgp activity to an increase in renal Pi reabsorption because, to our knowledge, there is no reliable method for measuring Pgp activity in the kidney in vivo or in BBMV. Pgp activity can be measured in proximal tubular cells in culture, but NPT2 expression rapidly decreases in these cells, thus precluding all conclusions.

In conclusion, we have shown three unrelated drugs that are known to inhibit Pgp activity, augmented renal Pi reabsorption in rats by increasing NPT2 expression in the apical membrane of proximal tubular cells. These findings may explain the effect of Dip on renal Pi threshold in humans, and lead to new insights into renal Pi transport regulation and treatment of renal Pi leak.

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